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The Lung Mast Cell: Its Physiology and Potential Relevance to Defense of the Lung

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The mast cell, located at mucosal surfaces and surrounding venules, is uniquely positioned to respond rapidly to insults to the host by mediating the development of a wide-ranging inflammatory response. Activation of the mast cell releases preformed granule-associated chemical mediators and generates *de novo* biologically active materials. The properties of the mast cell mediators permit development of both acute and prolonged inflammatory responses. The immediate response is characterized by edema and the delayed response by leukocyte infiltration and vascular damage.

The mast cell mediators responsible for these inflammatory events are characterized functionally. The vasoactive/smooth muscle reactive mediators include preformed histamine and serotonin and newly-generated platelet activating factor, slow reacting substance of anaphylaxis and prostaglandins. Chemotactic mediators include eosinophil-selective ECF-A and ECF-oligopeptides, neutrophil-selective NCF, and lipid chemotactic mediators with broad specificity. These factors induce directed migration and localization of leukocytes. The mast cell releases the structural proteoglycan, heparin, which is anticoagulant and inhibits complement. Released mast cell enzymes include chymotryptic and tryptic proteases, arylsulfatase, β-glucuronidase, and hexosaminidase. The proteolytic enzymes may activate inflammatory pathways while the others degrade ground substance. The capacity of the mast cell to enhance vascular permeability, to cause the influx of regulatory or inflammatory leukocytes, and to provide a variety of active enzymes permits regulation of inflammatory events at the site of tissue injury.

The mast cell, with its specific immunologic receptors, is positioned at portals of entry of potentially toxic or noxious exogenous material. In the lung the mast cell is located free in the bronchial lumen, in the bronchial mucosa in intra-epithelial locations, as well as in deeper perivenular collections (*1*, *2*). As it is present prior to entry of noxious agents and is thus freed from the requirements of mobilization and localization, the mast cell may be the sentinel cell for induction of local inflammatory responses. The development of inflammation by mast cells is consequent to the release of its content of potent biologic mediators as well as by its capacity to generate, *de novo*, active biologic materials from the local microenvironment. The knowledge that mediators are active *in vivo* follows from the *in vitro* definition of their biologic activities which relate to the known pathophysiology of inflammation. The inflammatory process induced by mast cell mediators is both acute and chronic and is subject to both positive and negative feedback controls inherent in the properties of the mediators themselves. It is the purpose of this review to delineate the physiology of the mast cell, the mediators generated upon mast cell activation and the regulation of mast cell-dependent inflammatory events.

Mast Cells

Mast cells are present in human lung at concentrations of 1-7 × 10^6 cells/g lung tissue (*3*). Each mast cell possesses, in addition to the ubiquitous subcellular organelles essential for all cell function, several hundred metachromatically staining granules each surrounded by a bilayer membrane. Lung mast cell granules possess a definite subgranular architecture (Fig. 1) of unknown functional significance. The

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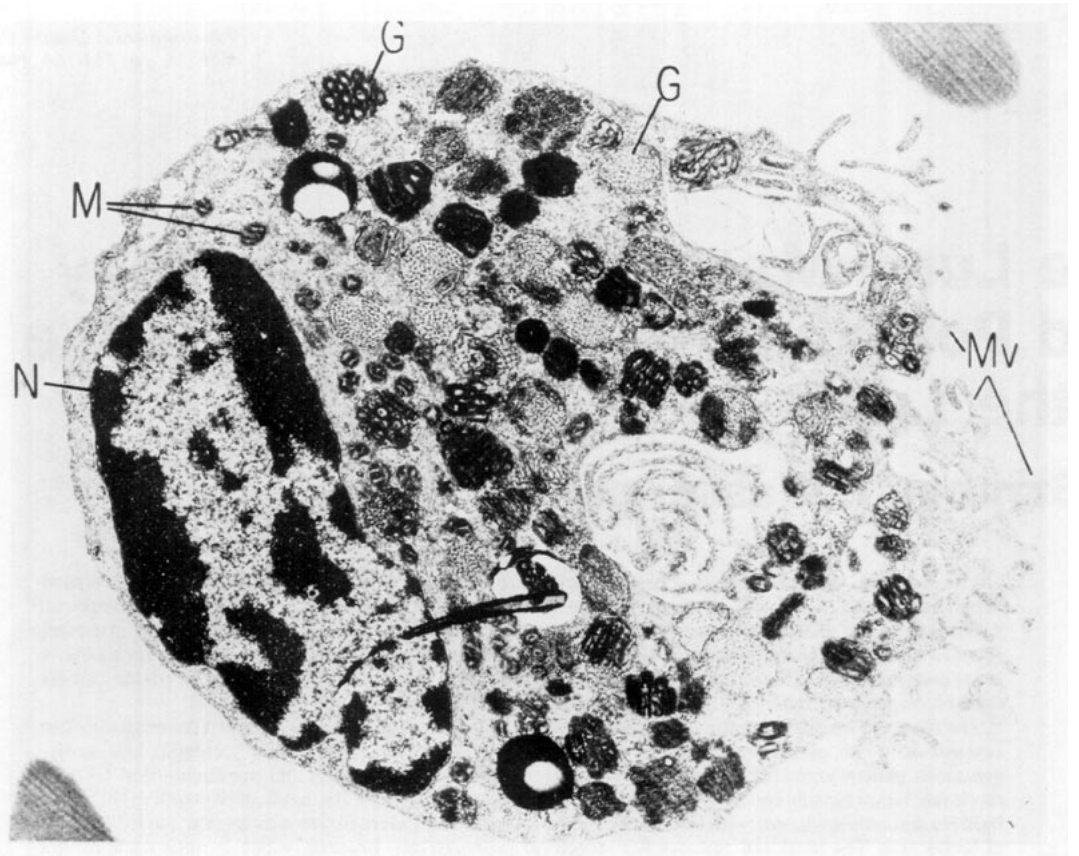

FIGURE 1. Electron micrograph of isolated human lung mast cell. Nucleus (N), membrane microvilli (MV), granule (G).

mast cell membrane is ruffled and possesses 50-300,000 receptors for IgE (*4*). IgE molecules belong to an immunoglobulin class defined by its activity in mediating immediate hypersensitivity reactions and by characteristic physicochemical properties. It comprises two heavy and light chains linked by disulfide bridges with a total molecular weight of 190,000. It is heat-labile, but does not form precipitates with specific antigen nor does it fix complement. The serum concentration of IgE is under genetic control, being higher in atopic than in normal individual(s). Papain digests IgE into three fragments, two of which (Fab) bind specific antigen and one (Fc) which binds to the specific receptors upon the mast cell surface. Receptors for the anaphylatoxins, C3a and C5a, fragments of the third and fifth components of complement, respectively, have been attributed to the mast cell upon functional criteria. In addition, mast cells may be degranulated by non-immunologic stimuli. Thus enzymes such as chymotrypsin, phospholipases A and C, and sialidase, ionophores, polycationic amines and proteins, radio-contrast media, and opiates may all effect mast cell degranulation. While it is generally assumed that atopic individuals exhibit their antigen-induced symptoms as a result of IgE-dependent mast cell activation, the demonstration of non-IgE mediated mechanisms for mast cell mediator release does not diminish the primacy of IgE in allergy but rather yields additional information on potential mechanisms for recruitment of mediators.

Activation and Degranulation of Mast Cells

The IgE-dependent degranulation of mast cells is initiated by the bridging of pairs of cell-bound IgE by specific antigen and terminates in 2 min. Bridging results in an alteration of the cell membrane which probably activates a surface membrane esterase. The cell membrane perturbation thus initiated is also associated with increased energy-dependent calcium entry into the cell. The calcium requirement for degranulation is related to the activation of the membrane serine esterase, and perhaps also to requirements for the expression of phospholipase activity, microfilament contraction, or membrane fusion. Degranulation also requires glucose and an intact glycolytic pathway. Finally, both ATP and calcium

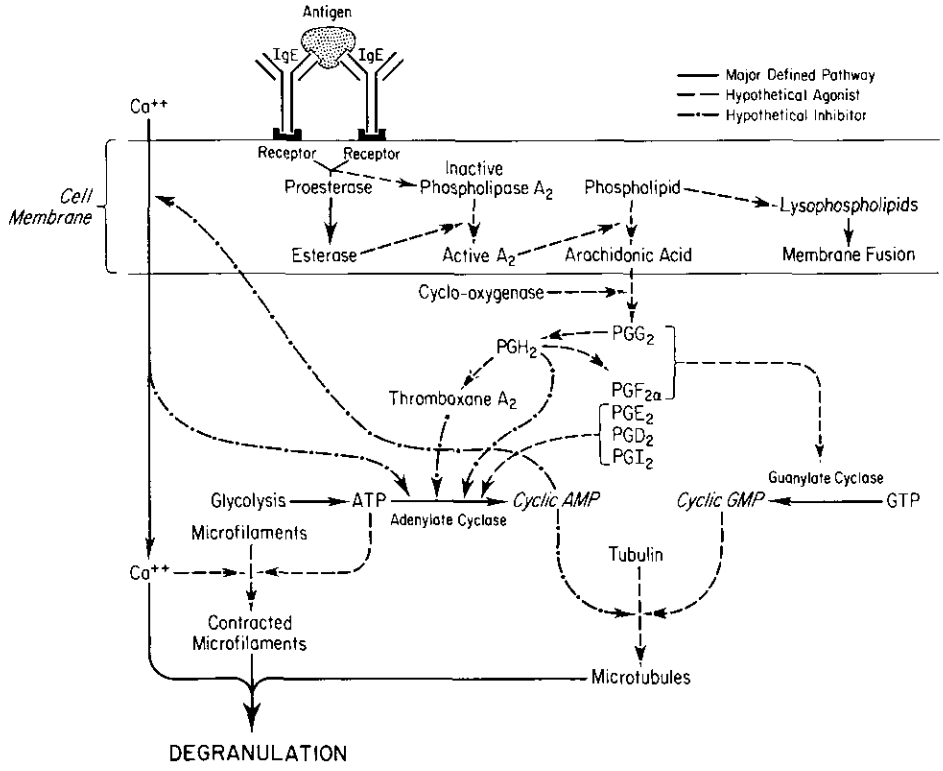

FIGURE 2. Schematic representation of proposed metabolic pathways relevant to antigen-induced degranulation of mast cells.

are required by a calcium-dependent ATPase, which probably activates contractile proteins. Following the ordered completion of these processes the perigranular membranes fuse with each other and with the cell membrane, and the granules are extruded (Fig. 2). Degranulation may be modulated at several steps in its sequence by endogenous or exogenous agents. Thus elevations in intracellular levels of cyclic adenosine 3′5′-monophosphate (cAMP), which may be induced by prostaglandins, histamine, and β-adrenergic agents, inhibit degranulation (*6*). Conversely, α-adrenergic or prostaglandin-induced falls in cAMP or rises in intracellular cyclic guanosine 3′5′-monophosphate (cGMP) following interaction with cholinergic stimuli or histamine, may enhance degranulation (*6*). The opposing actions of the cyclic nucleotides and the bidirectional control of degranulation by cAMP are felt to represent competitive effects upon a putative protein kinase which might regulate the state of cytoskeletal assembly. The supposition that cytoskeletal elements are important in human mast cell degranulation is supported by the inhibitory effects upon degranulation of colchicine and by the augmentation induced by cytochalasin B (*7, 8*).

Mast Cell-Dependent Mediators

Mast cells possess within their granules a variety of vasoactive, bronchospastic, and chemotactic mediators as well as a variety of active enzymes and structural proteoglycans (Table 1). Mast cell activation leads to the release of these preformed granular elements as well as to the generation of potent bronchospastic, vasoactive and chemotactic substances. Although best delineated in the case of the rat mast cell, it is likely that the human mast cell will be found similar in these regards.

Bronchospastic and Vasoactive Mediators

Histamine. Histamine, the product of decarboxylation of the amino acid histidine, is ionically bound to the proteoglycan-protein backbones of mast cell granules and is displaced by sodium exchange in the extracellular fluid (*9*). Histamine is catabolized by either oxidative deamination (histaminase) or by combined methylation and oxidative deamination (histaminase plus histamine *N*-methyl transferase (*10*). The pulmonary effects of histamine are expressed as both direct and reflex constriction of both

Table 1. Mast cell-dependent mediators.

Mediator	Structural characteristics	Function	Inhibition	Inactivation
		Bronchospastic and Vasoactive Activity		
Histamine (preformed)	β-Imidazolyl-ethylamine, MW 111	Contraction of smooth muscle Increase of vascular permeability Stimulation of suppressor T-lymphocytes Generation of prostaglandins Enhancement of (H_1) or inhibition (H_2) of chemotaxis Elevation of cAMP (H_2) and cGMP (H_1)	H_1 classical H_2 thiourea	Histaminase (diamine oxidase) or Histamine N-Methyl transferase
SRS-A (newly generated)	Acid hydrophilic sulfur-containing lipid (?), MW ~ 400	Contraction of smooth muscle Increased vascular permeability Synergistic with histamine Generation of prostaglandins	FPL-55712	Arylsulfatases A and B
Serotonin (preformed)	5-OH-tryptamine, MW 182	Contraction of some smooth muscle Increased vascular permeability	Hydroxyzine Cyproheptadine lysergic acid	Monoamine oxidase
PAF(s) (newly generated)	Lipid-like, MW 400-1000	Release of platelet amines Platelet aggregation Sequestration of platelets	Unknown	Phospholipases
Arachidonic acid metabolites PGD, PGE, PGI, $PGF_{2\alpha}$, HETE	C_{20} fatty acids (HHT C_{17} fatty acid)	Contract smooth muscle (PDG_2, $PGF_{2\alpha}$, TxA, PGG_2, PGH_2) Relax smooth muscle (PGE_2) Elevate cyclic AMP (PGE, PGD, PGI) Elevate cyclic GMP ($PGF_{2\alpha}$, PGG_2) Dose-dependent chemotactic attraction or eosinophils or neutrophils (HETE or HHT)	ETYA (cyclo-oxygenase Nonsteroidal Nonsteroidal anti-inflammatory	Several specific prostaglandin modifying engymes
		Chemotactic Activity		
ECF-A (preformed)	Val/Ala-Gly-Ser-Glu, MW 360-390	Chemotactic attraction and deactivation of eosinophils and neutrophils	Gly-Ser-Glu Val-Gly-Ser Ala-Gly-Ser	Amino-peptidase Carboxy-peptidase A
ECF-oligopeptides (preformed)	Peptides, MW 1300-2500	Chemotactic attraction and deactivation of eosinophils and neutrophils	Unknown	Unknown
NCF (preformed)	Neutral protein, MW > 750,000	Chemotactic attraction of deactivation of neutrophils	Unknown	Unknown
Lipid chemotactic factors (newly generated)	? HHT ? HETE ? other lipids	Chemotactic attraction of neutrophils and eosinophils Chemokinesis of neutrophils Deactivation of neutrophils	Unknown	Unknown
Histamine (preformed)	β-Imidazolyl-ethylamine, MW = 111	H_1 chemotactic and chemo-kinetic activation of eosinophils H_2 chemotactic and chemo-kinetic inhibition of neutrophils and eosinophils	H_1 classical H_2 thiourea	Histaminase (diamine oxidase) or Histaminase *N*-methyltransferase
		Structural Components		
Heparin (preformed)	Proteoglycan MW ~ 60,000	Anticoagulation Antithrombin III interaction Inhibition of complement activation	Protamine	Heparinase

Table 1 (Cont'd).

Mediator	Structural characteristics	Function	Inhibition	Inactivation
Chondroitin 4 and 6 sulfate (preformed)	Proteoglycan	Platelet factor IV interaction	Unknown	Chrondroitinase AC
Dermatan sulfate (performed)	Proteoglycan	Unknown	Unknown	Chondroitinase ABC
		Enzymes		
Chymase (preformed)	Protein MW = 29,000	Proteolysis with chymotryptic specificity	Serotonin Heparin Chymotrypsin inhibitors	Unknown
Arylsulfatase (preformed)	Proteins, MW = 100,000 (A) 60,000 (B)	Hydrolysis of SRS-A and various sulfate esters	PO_4, SO_4 product, substrate	Unknown
N,N-Acetyl-β-D-glucosaminidase (preformed)	Protein, MW = 158,000	Cleavage of glucosamine residues	Product	Unknown
Basophil (lung) kallikrein of anaphylaxis (preformed)	Protein, MW ≅ 400,000	Proteolysis with tryptic specificity Cleavage of kinin from kininogen Cleavage of Hageman factor	Trypsin inhibitors	Unknown
β-Glucuronidase	Protein, MW ≅ 300,000	Cleavage of glucuronide conjugates	Product	Unknown

large and small airways smooth muscle, thereby increasing airway resistance and decreasing compliance (*11*). It also dilates small radicles of the pulmonary vascular tree and increases the distance between endothelial cells of the venules, thereby increasing the potential for transudation of serum and for extravasation of leukocytes.

The biologic activities of histamine are expressed by its interaction with either of two specific classes of receptors on target cells. Those receptors designated H_1 predominate in skin and smooth muscle and are blocked by classic antihistamines, while H_2 receptors are selectively blocked by a group of compounds, including the thiourea derivatives, burami-mide, metiamide, and cimetidine (*12*). Pulmonary bronchoconstriction, vasodilation and increased cGMP are H_1 effects, while H_2 effects include inhibition of both human lymphocyte-mediated cytotoxicity and IgE-mediated histamine release due to elevation in cAMP content. Histamine inhibits chemotaxis through H_2 receptors, presumably also by stimulating adenylate cyclase and increasing cAMP.

Slow-Reacting Substance of Anaphylaxis (SRS-A). Although SRS-A remains structurally undefined, it is likely an unsaturated acidic sulfur-containing lipid of 300-500 daltons (*13*). It is active as a constrictor of peripheral airways to a much greater extent than of central airways (*14*) and causes vasodilation. Human lung tissue, dispersed pulmonary cells, and nasal polyps generate SRS-A during IgE-dependent immunologic reactions. Decreasing amounts of SRS-A are generated by increasingly pure human lung mast cell preparations suggesting that cell types other than mast cells alone are involved in its generation (*3*).

Presently the only acceptable assay for SRS-A employs the antihistamine-treated guinea pig ileum, but it is not specific for this mediator. Therefore, SRS-A must be further identified by its chromatographic properties, its susceptibility to inactivation by arylsulfatases, and its decreased assayability in the presence of a semispecific blocker, FPL55712.

Products of Arachidonic Acid Oxidation. In the human, products of arachidonic acid metabolism constitute the vast majority of prostaglandins and related compounds. Arachidonic acid mobilized from cell membrane phospholipids by the action of phospholipase A_2 is then either converted to prostaglandins and thromboxanes via a cyclooxygenase-dependent pathway or converted by a lipooxygenase to 12-L-hydroxy-5,8,10,14 eicosatetraenoic acid (HETE) and related compounds (Fig. 3). Several of these products have been described *in vitro* subsequent to noncytolytic activation of lung tissue and rat mast cells. Specifically,

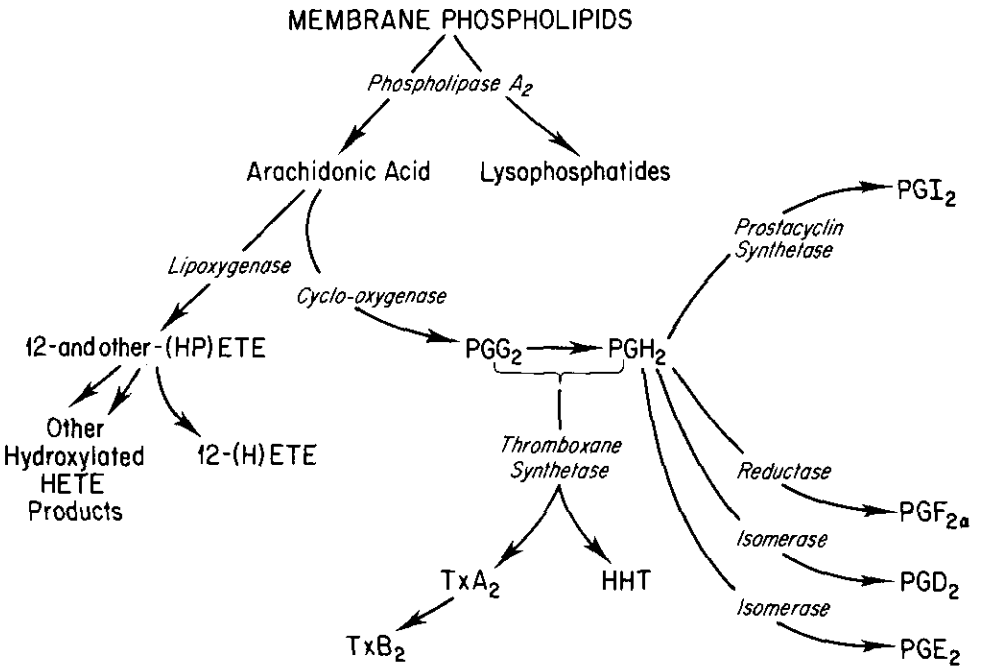

FIGURE 3. Pathways of generation of oxidative metabolites of arachadonic acid. Tx = thromboxane, PG = prostaglandin, HETE = hydroxy eicosatetraenoic acid, HHT = 12-L-hydroxy-5,8,10-heptadecatrienoic acid.

PDG_2, PGI_2, and HETE have been generated by isolated rat mast cells, whereas $PGF_{2\alpha}$ and PGE_2 are present after IgE-dependent activation of human lung tissue (*15, 16*). Guinea pig central airway smooth muscle is constricted by $PGF_{2\alpha}$, thromboxane A_2, and both cyclic endoperocides PGG_2. In the anesthetized dog, $PGF_{2\alpha}$ and PGD_2 both constrict central and peripheral airways. Additional pulmonary effects of generated prostaglandins are likely, as elevations in cAMP and cGMP may be consequent to their actions (*17*). While prostaglandins of the E and F series may be identified by radioimmunoassay, other arachidonic acid products must be isolated and measured by thin layer chromatography or gas chromatography-mass spectroscopy.

Platelet Activating Factors (PAF). PAF(s) are immunologically generated subsequent to IgE-dependent processes in human lung. Although PAF(s) are themselves neither bronchospastic nor vasoactive, they induce platelet release of serotonin. This monoamine contracts several smooth muscle preparations, but it is without apparent bronchospastic effect on the human. PAF(s) may also induce platelet aggregation and lead to the reversible sequestration of platelets in the lung. The best chemically described PAF(s) are apparently phospholipids, susceptible to inactivation by phospholipases (*18*). PAF from different sources are not identical, as shown by the inability of rabbit PAF from two sources to deactivate platelets to the biologic effects of each other and by the somewhat different phospholipase inactivation profiles of rabbit leukocyte and rat peritoneal PAF(s).

Chemotactic Mediators

Eosinophil Chemotactic Factor of Anaphylaxis (ECF-A). The first mast cell-associated chemotactic factor described, ECF-A, was identified in the anaphylactic supernatant of challenged guinea pig lung fragments and subsequently in the analogous human tissue and isolated mast cells. This factor is preferentially chemotactic for eosinophils. It also deactivates this cell type to further migration. A small peptide of about 400 daltons, ECF-A has also been identified preformed in human lung and isolated mast cells (*3*). This mediator from human lung has been structurally characterized as two acidic tetrapeptides with the amino acid sequence Val/Ala-Gly-Ser-Glu (*19*).

Intermediate Molecular Weight Eosinophil Chemotactic Peptides. In addition to low molecular weight ECF-A tetrapeptides, human lung contains chemotactic factors of MW 1200-2500 with specificity for eosinophil polymorphonuclear leukocytes (*20*). These factors are preformed, immunologically releasable and capable of deactivating eosinophils (*21*).

High Molecular Weight Neutrophil Chemotactic Factors (HMW-NCF). HMW-NCF has been described in rat mast cells, human lung fragments, and the serum of patients with cold urticaria (*22*) or asthma (*23*) following challenge with ice water or antigen inhalation, respectively. HMW-NCF has been characterized as a 750,000-dalton neutral protein which attracts and deactivates neutrophils *in vitro*. HMW-NCF appears to be a sensitive marker for mast cell activation as it is present in serum following challenge of allergic asthmatics with doses of antigen insufficient to induce bronchospasm.

Histamine. In addition to its smooth muscle directed actions histamine is active in modulating the migration of inflammatory leukocytes. Thus via an H_1 mechanism histamine enhances random and directed migration of eosinophils and neutrophils and is a weak chemotactic factor for human eosinophils. By its H_2 actions histamine inhibits both random and directed migration of neutrophilic and eosinophilic polymorphonuclear leukocytes (*24*).

Lipid Chemotactic Factors. The lipooxygenase product of arachidonic acid, HETE, has an ECF-A-like spectrum of activity. Unlike ECF-A, however, it increases eosinophil random migration and is a less potent chemotactic deactivator than ECF-A (*25*). Equivalent effects on neutrophils have also been described for the cyclooxygenase product 12-L-hydroxy-5,8,10-heptadecatrienoic acid (HHT) (*25*). A possibly related factor is a nonpolar lipid with chemotactic activity mainly for neutrophils which has been described in anaphylactic diffusates of rat mast cell-rich peritoneal cell preparations.

Structural Proteoglycans

The sulfated, metachromatic, mucopolysaccharide heparin has been identified in human lung and localized to the mast cells isolated from human lung tissue (*26*). Human lung heparin is a proteoglycan of approximately molecular weight 60,000 comprising a protein core to which are attached, by xylosyl-seryl linkages, glycosaminoglycan side chains of average molecular weight 20,000. Human also heparin interacts with human antithrombin III to accelerate anticoagulation. Heparin from other sources has been shown to inhibit generation of the convertase enzyme (C3bBb) responsible for amplification of cleavage of the third component of complement (C3) (*27*), to inhibit binding of the Clq fragment of complement to immune complexes (*29*), to inhibit action of the activated first component of complement ($\overline{C1s}$) upon its substrates, the fourth (C4) and second (C2) components (*29*), and to prevent binding of C2 to C4b (*30*). In addition, heparin has been shown to bind to platelet factor IV and to liberate lipoprotein lipase.

Granule-Associated Enzymes

Chymase. Chymase has been isolated from the mast cell of the rat and has been identified histochemically in the human mast cell (*31*). This enzyme is minimally active as a protease while stored in the granule, probably due to masking of the active site by heparin, but once freed its specific activity is comparable to that of pancreatic α-chymotrypsin. Chymotrypsin inhibitors have been shown to inhibit some inflammatory consequences of IgE-dependent activation in rabbit skin adding relevance to the mast cell location of this activity (*32*).

Lung Kallikrein of Anaphylaxis. A preformed enzyme of 400,000 molecular weight, termed lung kallikrein, is released subsequent to antigen challenge of IgE-sensitized human lung fragments (*33*). This activity can cleave kininogen to yield bradykinin and can activate Hageman factor. The released kinins may contract bronchial smooth muscle and increase vascular permeability. Kinins have also been noted to induce bronchospasm when inhaled by asthmatic humans and have been detected in the serum of a single patient sustaining an acute asthma attack. Activation of Hageman factor with the induction of the clotting and fibrinolytic cascades could also be reasonably expected to ensue upon release of lung kallikrein.

Arylsulfatase. Arylsulfatase has been identified in human lung mast cells (*3*). Arylsulfatase A and B enzymes can inactivate SRS-A and furthermore, arylsulfatase A is immunologically released with the mast cell granule in the rat (*34*).

Other Enzymes. β-Glucuronidase and *N*-acetyl-β-D-glucosaminidase (hexosaminidase) have been identified in human lung and in rat mast cells and both are released upon immunologic activation of the rat mast cell (*35*). These enzymes in concert with arylsulfatase, when obtained from sources other than mast cells, have been demonstrated to degrade ground substance mucopolysaccharides.

Mediator Interactions

Although the mediators of immediate type hypersensitivity have been identified and assessed for their effects as isolated factors some understanding of their interactions is available. Thus the spasmogenic effects of histamine and SRS-A are synergistic and in addition the presence of either directly induces prostaglandin generation. The interactions of ECF-A, histamine, and ECF-oligopeptides are not fully delineated but it is known that histamine in high concentrations inhibits and in low concentrations enhances ECF-A-induced eosinophil chemotaxis. In addition, the N and C terminal tripeptides of ECF-A

are reversible and irreversible inhibitors of eosinophil chemotaxis, respectively (*37*). In addition, PGE_1 enhances and $PGF_{2\alpha}$ inhibits neutrophil chemotaxis probably by their actions upon cyclic nucleotide concentrations. However, given the number and complexity of mast cell-dependent mediators other, potentially critical, biologic interactions await elucidation.

Determinants of Activity of Mast Cell Mediators

Following activation of mast cells by IgE-dependent or other mechanisms, the panoply of mediators generated and released and the myriad of their potential interactions provides the substrate for induction of inflammatory events. The putative role for the mast cell and its mediators in both the homeostatic as well as pathophysiologic induction of inflammation in the lung is strengthened by the location of the mast cell at the respiratory surface. Thus the interaction of surface and intraluminal mast cells with antigen or noxious agents could, by the action of locally released permeability factors, lead to alterations of the respiratory epithelial barrier thereby permitting access to the large number of deeper situated mast cells. Of more direct relevance has been the *in vivo* demonstration that solely mast cell-dependent events can indeed cause both immediate and more persistent inflammatory processes. The biphasic response of airways to inhaled antigen may be comparable to the biphasic cutaneous response to IgE-dependent mast cell activation (*38, 39*). In the cutaneous model a wheal-and-flare response reflects altered vascular permeability at that site, whereas later inflammatory events are accompanied by an intense cellular infiltration (*39*). Immunochemical studies have not revealed the participation of immune complexes or complement during this later inflammatory phase (*39*). Elicitation of both phases by purely IgE-dependent mechanisms indicates that mast cell activation can contribute to subacute and chronic as well as to acute pathobiologic processes. This fact is central to according the mast cell an important role in asthma, since this disease is characterized by acute exacerbations superimposed on chronic hyperirritability of the airway. Whether continued alteration in the threshold of airway response is due to mast cell mediators directly or to cellular infiltration is not established, but mast cell-derived mediators could be critical by either route alone or in combination.

The mechanism by which mast cell mediators might provoke such biphasic inflammatory responses is depicted in Figure 4. The mast cell dependent generation of bronchospastic and vasoactive mediators establishes a local vasodilatory or humoral phase of inflammation while the release of chemotactic mediators provokes a cellular phase of inflammation. The humoral phase, apparent within minutes, would lead to egress from the circulation of immunoglobulin and complement as well as fibrinolytic, procoagulant and kinin-generating proteins. This response could be expected to be rapidly beneficial to the host by aiding removal of invading microorganisms by localizing and removing noxious agents, and by facilitating leukocyte migration through venular disconnections. On the other hand,

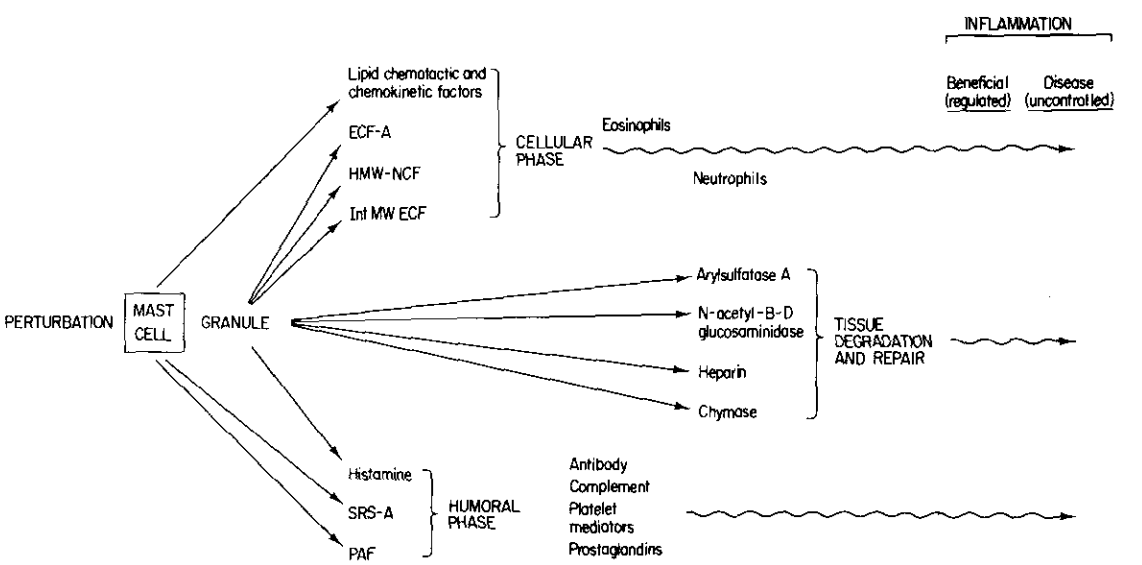

FIGURE 4. Schematic representation of mast cell dependent humoral and cellular inflammatory events.

an unregulated humoral phase might be expressed in disease as acute exacerbations of bronchospasm, alterations in tracheobronchial mucus flow or consistency, or as upper airway obstruction due to edema. The generation and release of chemotactic factors would, in a period of several hours, be expected to call to the local inflammatory focus both eosinophilic and neutrophilic polymorphonuclear leukocytes. These cells could prove beneficial not only by their phagocytic function but also by their ability to inactivate mediators (see below). Conversely, if accumulation of leukocytes were uncontrolled the pathobiologic consequences of tissue infiltration with leukocytes would ensue. Clinically, such infiltration might be seen in the inflammatory bronchial epithelium of asthmatic patients, in vasculitis, or perhaps, via the action of leukocytic lysosomal enzymes, in the destruction of lung tissue and the induction of fibrosis. By their capacities to deactivate leukocytes to further chemotactic activation, mast cell chemotactic mediators might also inhibit localization of leukocytes. In cold urticaria, following mediator release by experimental challenge, circulating neutrophils are rendered unresponsive to *in vitro* chemotactic stimulation. This effect is time limited but persists beyond the period in which measurable quantities of mediators are noted (*40*). Such an inhibitory action of chemotactic factors could prove beneficial by blunting exuberant inflammation or if prolonged or ill-timed might be harmful by preventing adequate host response to local insult. The release of mast cell proteases and lysosomal enzymes may themselves lead to alterations in ground substances and to activation of such protein inflammatory cascades as complement, fibrinolysis and coagulation. These processes would amplify inflammatory events and might prove beneficial to the elimination of microorganisms or toxic agents but might also, if unregulated, lead to tissue destruction, chronic inflammation and fibrosis.

Regulation of Mast Cell Mediator Release and Activity

The events which lead to the generation and release of mast cell mediators and thereby to the elicitation of mast cell dependent inflammatory events are subject to regulation at several critical points. Thus, activation of the mast cell, the secretion of granules, the release of the preformed mediators and generation of unstored mediators, the effect of mediators upon target cells and finally the persistence in tissue of the mediators are all under biologic control (Fig. 5).

The extent and effect of activation of the mast cell is controlled by regulation of access of the eliciting

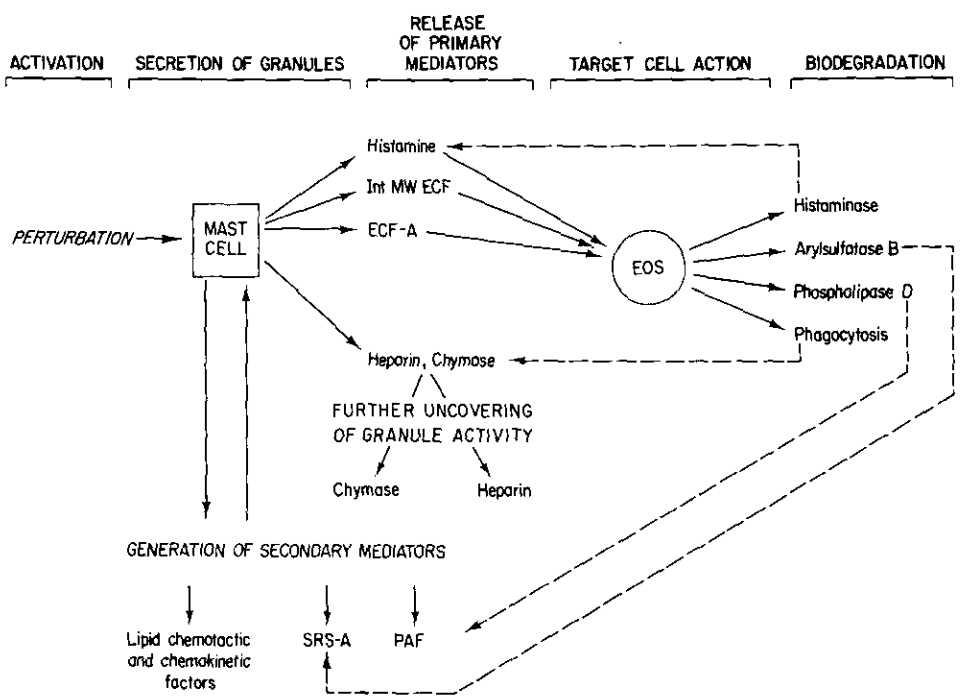

FIGURE 5. Schematic representation of endogenous regulation of mediator expression with particular emphasis on the role of the eosinophil.

agent to this cell, the amount of specific IgE antibody bound to the mast cell surface and by the intensity of the stimulus. The mast cell itself might alter local permeability allowing increased antigen contact, or by the H_2 action of histamine may suppress lymphocyte recognition of antigen and thereby prevent immune response to invaders. In addition the ratio of specific mediator generated is dependent upon the intensity of the activating stimulus as limited stimulation of the mast cell leads to intracellular accumulation of SRS-A without release of SRS-A or histamine (*41*). Intracellular mast cell arylsulfatases may also control mediator generation by degrading SRS-A prior to its release.

Subsequent to activation of the mast cell the secretion of granules is under control of cyclic nucleotides as noted above. Of direct relevance is the possibility that the mast cell itself, by the mediator histamine via an H_2 action and prostaglandins E_2, D_2, and I_2 may directly elevate cAMP and thereby inhibit granule secretion. Conversely, the H_1 action of histamine to elevate cGMP and that of $PGF_{2\alpha}$ to lower cAMP levels might augment mediator release.

Following secretion of the mast cell granule, the release of preformed mediators and thus the full expression of their activity is dependent upon the independent solubilities of each of the mediators. Thus β-glucuronidase, arylsulfatase A, histamine, ECF-A, and ECF-oligopeptides are fully soluble in physiologic buffers, hexosaminidase requires 0.5*M* NaCl for full elution from the granule and chymase-heparin complex requires 1.0*M* NaCl for dissolution (*34, 42*). The relevance of granule binding is emphasized by the marked increase in proteolytic activity of the chymase following its elution from the granule. Although the mechanism(s) in which the mast cell granule is solubilized *in vivo* remain unknown, it is clearly an important step in regulating the activity of mast cell inflammatory mediators.

Regulation of the action of mast cell mediators upon target cells may derive from alterations in cyclic nucleotide concentrations, as noted above, or by the interaction of several mediators, or their metabolites with the same target cell. Thus, histamine may be additive or inhibitory to ECF-A action upon eosinophils depending upon the ratio of the two activities present. In addition, the constituent tripeptides of ECF-A are inhibitory to induction to eosinophil chemotaxis by ECF-A. As noted above, mediators may also be synergistic on smooth muscle as exemplified by SRS-A and histamine action.

The regulation of generation of unstored mediators has not been fully elucidated but data suggest that the local microenvironment is critical to their generation. For example, SRS-A generation is maximal in mixed rather than in pure mast cell populations (*3*), and SRS-A, PAF and lipid chemotactic factors can all be generated from monunuclear leukocyte populations. The ratios of critical target cells thus may be crucial to the amount and type of mediator generated.

Finally, the persistence of the effect of mediators is also regulated. While this may reflect the limited availability of key mediators, probable tachyphylaxis to some, and mediator clearance by excretion, it is also affected by enzymatic inactivation of these biologic activities. As many mast cell-derived chemotactic mediators are eosinophilotactic, the eosinophil content of mediator-inactivating enzymes may provide a feedback control of mediator effects (Fig. 5). Notably, the eosinophil contains histaminase, arylsulfatase B, and phospholipase D, which inactivate histamine, SRS-A, and PAF, respectively. SRS-A is inactivated not only by extracted eosinophil arylsulfatase, but also by the enzyme-rich resting granulocyte, presumably after cellular uptake of the mediator. Some other cells also contain mediator-inactivating enzymes. Neutrophils contain histaminase, and at least one mononuclear cell population is rich in histamine methyltransferase. Interestingly, both mast cells and basophils contain arylsulfatase.

Inactivation of arachidonic acid metabolites is complicated, as some intermediate metabolic products are biologically active. Thus PGD_2 and thromboxane A_2 derived from the cyclic endoperoxides are potent bronchoconstrictors. However, most stable catabolic end products of arachidonic acid metabolism, such as thromboxane B_2 (from thromboxane A_2) and PG-6-keto-$F_{1\alpha}$ (from PGI_2) have little or no defined biologic activity.

Conclusion

The mast cell-derived preformed and newly generated mediators are released by IgE-dependent and independent mechanisms, and are biologically available during inflammatory events. The relevance of these mediators to allergic disease has been derived from studies of the pathophysiologic alterations induced by the individual mediators, identification of the mediators in tissue or biologic fluids of patients experiencing allergic reactions, and the known pathophysiology of the various atopic diseases.

Mediators of immediate hypersensitivity not only possess the ability to induce immediate tissue responses such as a wheal and flare, anaphylaxis, or rapid-onset brief-duration alterations in pulmonary function, but may also mediate a prolonged inflammatory response. The fact that IgE and mast cells are relevant to prolonged inflammatory events has been

documented by passive transfer with isolated IgE, of delayed inflammatory responses in skin and by the dependence upon IgE antibody for similar delayed alterations in pulmonary mechanics following inhalation of antigen. In lung, these delayed responses are prevented by pretreatment with disodium cromoglycate, which supports the central role of the mast cell. Histopathologic assessment of delayed responses reveals an influx of neutrophils, eosinophils, basophils, lymphocytes, and mononuclear leukocytes, the deposition of fibrin, and vascular abnormalities which may progress to frank vasculitis (*39*). Although some of the mediators responsible for the early and later phases of the IgE-mast cell reaction can be surmised from the kinetics of their *in vitro* effects, their absolute identification and participation in disease requires further definition.

The postulated role of the mast cell and its mediators in inflammation may provide insight not only into the clinical evolution of some allergic disorders such as progression of seasonal to perennial asthma, but also into the local homeostatic regulation of the lung environment and thereby the defense of the lung. Although much remains to be clarified, the rapidly expanding understanding of target cell activation together with the identification of mast cell derived mediators provides a framework for definition of the complex processes that provide pulmonary defenses.

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